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(54) Title: TCL-1b GENE AND PROTEIN AND RELATED METHODS AND COMPOSITIONS**(57) Abstract**

The *TCL1* gene family, located on the human chromosome at the 14q32.1 locus, are implicated in the development of T-cell malignancies. The present invention discloses the identification and characterization of a new member of this gene family, the *TCL-1b* gene. The *TCL-1b* gene sequence is expressed at very low levels in normal bone marrow and peripheral lymphocytes, but is activated in T-cell leukemia and lymphoma by rearrangements of the 14q32.1 locus. The present invention relates to the identification of these chromosome 14 abnormalities, and methods for detecting and treating any T-cell malignancies that develop, as well as preventing the development of these T-cell malignancies.

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TCL-1b GENE AND PROTEIN AND RELATED METHODS AND COMPOSITIONS

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CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority under 35 USC §119 based upon U.S. Provisional Patent Application No. 60/124,714 filed March 15, 1999.

FIELD OF THE INVENTION

The present invention relates to the field of molecular biology, more particularly to the isolation and characterization of a third member of the *TCL1* gene family, specifically *TCL-1b*, also activated by chromosomal rearrangements in T cell leukemias.

BACKGROUND OF THE INVENTION

There is a close association between particular chromosomal abnormalities, e.g., chromosomal translocations, inversions, and deletions, and certain types of malignancy indicating that such abnormalities may have a causative role in the cancer process. Chromosomal abnormalities may lead to gene fusion resulting in chimeric oncoproteins, such as is observed in the majority of the tumors involving the myeloid lineage. Alternatively, chromosomal abnormalities may lead to deregulation of protooncogenes by their juxtaposition to a regulatory element active in the hematopoietic cells, such as is observed in the translocation occurring in the

lymphocytic lineage (Virgilio, et al., 1993, *Proc Natl Acad Sci USA* 90:9275-9279).

Non random chromosomal translocations are characteristic of most human hematopoietic malignancies (Haluska, et al., 1987, *Ann Rev Genet*, 21:321-345) and may be involved in some solid tumors (Croce, 1987, *Cell* 49:155-156). In B and T cells, chromosomal translocations and inversions often occur as a consequence of mistakes during the normal process of recombination of the genes for immunoglobulins (Ig) or T-cell receptors (TCR). These rearrangements juxtapose enhancer elements of the Ig or TCR genes to oncogenes whose expression is then deregulated (Croce, 1987, *Cell* 41:155-156). In the majority of the cases, the rearrangements observed in lymphoid malignancies occur between two different chromosomes.

The *TCL-1* locus on chromosome 14 band q32.1 is frequently involved in the chromosomal translocations and inversions with the T-cell receptor genes observed in several post-thymic types of T-cell leukemias and lymphomas, including T-prolymphocytic leukemias (T-PLL) (Brito-Babapulle and Catovsky, 1991, *Cancer Genet. Cytogenet.* 55:1-9), acute and chronic leukemias associated with the immunodeficiency syndrome ataxia- telangiectasia (AT) (Russo, et al., 1988, *Cell* 53:137-144; Russo et al., 1989, *Proc Natl Acad Sci USA*, 86:602-606), and adult T-cell leukemia (Virgilio, et al., 1993, *Proc Natl Acad Sci USA*, 90:9275-9279).

The *TCL1* oncogene on chromosome 14q32.1 is also involved in the development of chronic T-cell leukemia in humans (T-CLL) and is activated in these leukemias by juxtaposition to the T-cell receptor α/δ locus, caused by chromosomal translocations, t(14;14)(q11;32), t(7;14)(q35;q32), or inversions inv(14)(q11;q32). Normally *TCL1* expression is observed in early T-cell progenitors (CD4⁺CD8⁺CD3⁺) and lymphoid cells of the B-cell lineage: pre B-cells and immature IgM expressing B-cells. Introduction of a *TCL1* transgene under the control of a *lck* promoter caused mature T-cell leukemia in mice. (Virgilio, et al., 1998, *Proc Natl Acad Sci USA*, 95:3885-3889).

However, some cases of T-cell malignancies with abnormalities such as gene amplification at 14q32.1 did not show activation of the *TCL1* expression, suggesting that perhaps an additional oncogene may be located in 14q32.1. The

second member of the *TCLI* gene family, *MTCPI*, is located at Xq28 and activated in rare cases of mature T-cell leukemia with a t(X;14)(q28;q11) translocation. The present invention involves the isolation and characterization of the third member of the *TCLI* gene family, *TCL1b*, located at 14q32.1 and also
5 activated by rearrangements at 14q32.1 in T-cell leukemias.

Rearrangements of the *TCL-I* locus at chromosome 14q32.1 are unique, in that the other locus involved in these rearrangements, namely the *TCR* α/δ locus, is also on chromosome 14 at subband q11 (Croce, et al., 1985, *Science* 227:1044-1047; Isobe, et al., 1988, *Proc Natl Acad Sci USA*, 85:3933-3937). For
10 this reason, the rearrangements observed cytogenetically are either chromosomal inversions, inv(14) (q11;q32), involving only one of the chromosomes 14 or translocations involving both chromosomes 14 such as the t(14;14) (q11;q32), or more rarely, the t(7;14) (q35;q32) involving the *TCR* β locus at 7q35 (Isobe et al., 1988, *Proc Natl Acad Sci USA*, 85:3933-3937). Several of the breakpoints at
15 14q32.1 involved in these translocations have been cloned and characterized (Russo, et al., 1988, *Cell*, 51:137-144; Baer, et al., 1987, *Proc Natl Acad Sci USA*, 84:9069-9073; Mengle-Gaw et al., 1987, *EMBO*, 1:2273-2280; Bertness et al., 1990, *Cancer Genet Cytogenet*, 44:47-54).

The *TCL-I* locus, a chromosomal region of approximately 350 kb as
20 determined by placement of translocation breakpoints on the long range genomic map, has recently been cloned (Virgilio, et al., 1993, *Proc Natl Acad Sci USA*, 90:9275-9279). The involvement of such a large region in translocation events suggests that activation of the putative *TCL-I* gene may occur from a distance of many kilobases, as previously observed for the *BCL-1/CCND1* gene in mantle cell
25 lymphoma (Tsujimoto, et al., 1984, *Science*, 224:1403-1406; Rosenberg, et al., 1991, *Proc Natl Acad Sci USA*, 88:9638-9642; Withers, et al., 1991, *Mol Cell Biol*, 11:4846-4853; Motokura and Arnold, 1993, *Genes Chrom & Cancer*, 7:89-95) and the *MYC* oncogene in Burkitt lymphoma (Dalla-Favera, et al., 1982, *Proc Natl Acad Sci USA*, 79:7824-7827; Nishikura, et al., 1983, *Proc Natl Acad*
30 *Sci USA* 80:4822-4826) and in acute T-cell leukemia (Erikson, et al., 1986, *Science*, 232:884-886).

There remains an unfulfilled need to fully isolate and characterize the third member of the *TCL-1* gene family, *TCL1b*. The identification of an additional oncogene that is associated with chromosomal abnormalities causing T-cell leukemias and lymphomas further expands the efficacy by which a diagnostic and therapeutic/prophylactic reagent will detect, treat, and prevent such disease states. The present invention fulfills this need by the identification and characterization of the *TCL1b* gene.

Citation of references herein above shall not be construed as an admission that such references are prior art to the present invention.

SUMMARY OF THE INVENTION

The *TCL1* gene family is implicated in the development of T-cell malignancies. The present invention discloses the identification and characterization of a new member of this gene family, the *TCL-1b* gene. The present invention relates to the nucleotide sequences of *TCL1b* and amino acid sequences of their encoded Tc1b proteins, as well as derivatives and analogs thereof, and antibodies thereto. The present invention further relates to nucleic acids hybridizable to or complementary to the foregoing nucleotide sequences, as well as equivalent nucleic acid sequences encoding a Tc1b protein.

The present invention relates to expression vectors encoding a Tc1b protein, derivative or analog thereof, as well as host cells containing the expression vectors encoding the Tc1b protein, derivative or analog thereof.

The present invention further relates to the use of *TCL1b* genes and their encoded proteins as diagnostic and therapeutic tools for the detection and treatment of disease states associated with chromosomal abnormalities, specifically abnormalities at 14q32.1. In one embodiment of the present invention the use of nucleotide sequences of *TCL-1b* genes and amino acid sequences of their encoded Tc1b proteins, respectively, are used as diagnostic reagents or in the preparation of diagnostic agents useful in the detection of disease states, such as T-cell

leukemias and lymphomas, associated with chromosomal abnormalities, in particular at 14q32.1, and/or increased levels of expression of the Tc11b protein.

The invention further relates to the use of nucleotide sequences of *TCL-1b* genes and amino acid sequences of their encoded Tc11b protein, respectively, as therapeutic/prophylactic agents in the treatment/prevention of disease states, such as T-cell leukemias, associated with chromosomal abnormalities, in particular at 14q32.1, and/or increased levels of expression of the Tc11b protein.

The *TCL-1b* genes and Tc11b protein sequences disclosed herein, and antibodies thereto, are used in assays to diagnose T-cell leukemias and lymphomas associated with chromosomal abnormalities, and/or increased expression of Tc11b protein.

The Tc11b protein, or derivatives or analogs thereof, disclosed herein, are used for the production of anti-Tc11b antibodies, respectively, which antibodies are useful diagnostically in immunoassays for the detection or measurement of Tc11b protein, respectively, in a patient sample.

Another aspect of the present invention relates to methods of treatment of diseases or conditions associated with chromosomal abnormalities and/or increased expression of Tc11b proteins. Abnormalities of chromosome 14, such as inversions and translocations, particularly at 14q32.1, are associated with T-cell leukemias and lymphomas. *TCL-1b* gene sequences and their protein products are used therapeutically in the treatment of disease states associated with chromosome 14 abnormalities. Anti-Tc11b antibodies are used therapeutically, for example, in neutralizing the activity of an overexpressed Tc11b protein, respectively, associated with disease.

Oligonucleotide sequences, including antisense RNA and DNA molecules and ribozymes, designed to inhibit the transcription or translation of *TCL-1b* or mRNA, are used therapeutically in the treatment of disease states associated with increased expression of Tc11b.

Proteins, peptides and organic molecules capable of modulating activity of Tc11b are used therapeutically in the treatment of disease states associated with aberrant expression of Tc11b.

The present invention further relates to therapeutic compositions comprising Tc11b proteins, derivatives or analogs thereof, antibodies thereto, nucleic acids encoding the Tc11b proteins, derivatives or analogs, and *TCL-1b* antisense nucleic acid.

- 5 The present invention further relates to methods of production of the Tc11b proteins, derivatives and analogs, such as, for example, by recombinant means.

DESCRIPTION OF THE DRAWINGS

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Figure 1. Sequence comparison of Tc11, Tc1-1b and Mtcp1. Identities are shown in black boxes, similarities are shown in shaded boxes. For Tc11 and Mtcp GenBank accession numbers are X82240 and Z24459, respectively.

- 15 **Figure 2** Figure 2. Genomic organization of the *TCL1* and *TCL1b* genes. Vertical arrows refer to cloned 14q32.1 breakpoints. Restriction sites are given for BssHII (B), ClaI (C), EagI (E), SfiI (F), KspI (K), MluI (M), NotI (N), NruI (R) and SalI (S). Solid boxes represent *TCL1* and *TCL1b* exons.

- 20 **Figure 3.** Northern analysis of the *TCL1* and *TCL1b* genes. (A). Human immune system Northern blot. Lanes 1-6: spleen; lymph node; thymus; peripheral blood leukocyte; bone marrow; fetal liver. (B). Human cancer cell line Northern blot. Lanes 1-8: promyelocytic leukemia, HL-60; Hela cells; chronic myelogenous leukemia, K-562; T-lymphoblastic leukemia, MOLT-4; Burkitt's lymphoma Raji; colorectal adenocarcinoma, SW480; lung carcinoma, A549; melanoma, G361. (C). Lanes 1-6: Burkitt's lymphoma Raji; Burkitt's lymphoma Daudi; Burkitt's lymphoma CA-46; SupT11; bone marrow; placenta. (D). Lane 1: bone marrow; lanes 2-7, EBV transformed lymphoblastoid cell lines: Ado-1471; Ado-1476; Ado-1701; Ado-1727; Ado-2069; Ado-2199; lane 8: CA-46. (A-D). Top, TCL1b probe; 30 middle, Tc11 probe; bottom, actin probe.

Figure 4. RT-PCR analysis of the *TCL1* and *TCL1b* genes. (A). Normal human tissues. Lanes 1-23: heart; liver; brain; muscle; placenta; kidney; lung; pancreas; spleen; lymph node; thymus; tonsil; peripheral blood lymphocytes (PBL); fetal liver; fetal brain; fetal lung; fetal kidney; fetal heart; fetal skeletal muscle; fetal spleen; fetal thymus; negative control. (B) Lanes 1-4, T cell PLL samples: 3047; 3046; 3050; 3048. Lanes 5-6: bone marrow; PBL. (A-B). Top, *TCL1b* primers; middle, *TCL1* primers; bottom, control G3PDH primers.

10 DESCRIPTION OF THE INVENTION

Methods

Cell lines.

Cell lines, except EBV transformed lymphoblastoid cell lines, were obtained from ATCC (Rockville, MD) and grown in RPMI media with 10% fetal bovine serum. Lymphoblastoid cell lines were made from peripheral blood lymphocytes of patients with Alzheimer's disease by transformation with Epstein-Barr virus (EBV) as previously reported (Gritti, C., et al., 1998, *Blood*, 92:368-373).

20 *Northern, Rapid Amplification of cDNA Ends (RACE) and Reverse Transcription-PCR (RT-PCR) analysis.*

These experiments were carried out as previously described (Pekarsky, Y., et al., 1998, *Proc Natl Acad Sci USA*, 95:8744-8749) with the following exceptions. Human bone marrow and placenta mRNAs, human immune system and human cancer cell line Northern blots were purchased from Clontech (Palo Alto, CA). Each line on Figure 3C and D contains 3 mg PolyA+ RNA. PCR shown on Figure 4A was carried out for 25-35 cycles using Multiple Tissue cDNA Panels (Clontech) and manufacturer's protocol. Primers were: top panel, *TCL1*, GGCAGCTCTACCCCGGGATGAA (SEQ. ID. NO: 1); and *TCL39*, (SEQ. ID. NO: 1); ACAGACCTGAGTGGGACAGGA, (SEQ. ID. NO: 2); middle panel, *TCLB* TCCTCCTTGGCAGGAGTGGTA (SEQ. ID. NO: 3); and *TCLC*, CAGTTACGGGTGCTCTTGCCT, (SEQ. ID. NO: 4); lower panel, control 3' and

5' RACE G3PDH primers (Clontech). Fig. 4B, middle and bottom panels, primers were the same as above. Figure 4B, top panel. PCR was carried out for 22 cycles with primers TC8, ATGGCCTCCGAAAGCTTCTGTG, (SEQ. ID. NO: 5); and TC39. 0.1 ml of the reaction was used for the second PCR with nested primers TC10, TGGTCGTGCGGTTCAATCCCT, (SEQ. ID. NO: 6); and TC5, AATCTGGCCATGGTCTGCTATTTC, (SEQ. ID. NO: 7); for 15 cycles. RACE primers were: TC1 (for 3' RACE) and TC5 (for 5' RACE).

Pulsed-Field Gel Electrophoresis (PFGE) analysis and chromosomal localization.

PFGE analysis was performed as described (Pekarsky, Y, et al., 1998, *Cancer Res*, 58:3401-3408), except pulse time was 1-6 second for 11 hours. Chromosomal localization of the *TCL1b* gene was carried out using GeneBridge 4 radiation hybrid mapping panel (Research Genetics, Huntsville, AL) according to the manufacturer's protocol. Primers were TC1 and TC4, TGCTAGGACCAGCTGCTCCATAGA, (SEQ. ID. NO: 8).

Results

*Identification of the *TCL1b* gene.*

In some mature T-cell leukemias with chromosomal abnormalities at 14q32.1, activation of the *TCL1* gene at 14q32.1 was not observed (Takiwaza, J., et al., 1998, *Jpn J Cancer Res*, 89:712-718; Sakashita, et al., 1998, *Leukemia*, 12:970-971). To investigate the possibility that other, unknown *TCL1* family member(s) may be involved, we searched the EST database for sequences homologous to the *TCL1* and *MTCPI* gene products. A single EST (accession number AA689513) was found to be homologous, but not an exact match to both genes. Thus, a ~1.2 kb full length cDNA was isolated (SEQ. ID. NO: 9); using 5' and 3' RACE procedure and human testis mRNA as a cDNA source. The 1.2 kb *TCL1b* cDNA encodes a 14 kDa protein of 128 amino acids (SEQ. ID. NO: 10); (Figure 1). It contains a starting ATG codon at position 28 within a perfect Kozak consensus sequence. The *Tcl1b* protein has a 14 amino acid insertion compared to

the *Tcl1* and *Mtcp1* proteins (Figure 1); it is 30% identical and 60% similar to *Tcl1*, and 36% identical and 63% similar to *Mtcp1* (Figure 1).

A radiation hybrid mapping panel (GeneBridge 4) was used to determine the chromosomal localization of the human *TCL1b* gene. By analysis of PCR data at the MIT database (<http://www-genome.wi.mit.edu>), the *TCL1b* gene was localized to 3.05 cR from the marker D14S265, at 14q32. A *TCL1b* pseudogene and localized it to 5q12-5q13. The *TCL1b* pseudogene does not have the initiating ATG or introns and has a stop codon in the middle of the open reading frame.

TCL1 and *TCL1b* are both located at 14q32, therefore, a determination was made as to whether *TCL1* and *TCL1b* are physically linked. The human bacterial artificial chromosome (BAC) library and found several BAC clones containing *TCL1* and *TCL1b*. The *TCL1b* gene (SEQ. ID. NO: 11); is 6.5 kb in size and contains 4 exons of 189, 171, 69 and 697 bp respectively (Figure 2), but only the first three exons are coding. Pulsed field analysis of the positive BAC clone with both probes revealed that the *TCL1* and *TCL1b* genes have opposite directions of transcription and are separated only by 16 kb (Figure 2). Both genes are located in the ~160 kb region between previously published two sets of breakpoints observed in T-cell acute lymphoblastic leukemia (ALL) cases with translocations or inversions at 14q32.1 (Virgilio, L., et al., 1994, *Proc Natl Acad Sci USA*, 91:12530-12534; Virgilio, L., et al., 1993, *Proc Natl Acad Sci USA*, 90:9275-9279).

Expression of TCL1b gene and its activation in T-cell malignancies.

Because of the similarities between the *TCL1* and *TCL1b* genes in their structure, sequence, and location, it seemed possible that they would exhibit similar expression patterns. To verify this, we carried out a series of Northern and RT-PCR experiments (Figures 3 and 4). Northern analysis in normal tissues was mostly negative for *TCL1b* (Figure 3A), except that the 1.2 kb transcript was detected after several days exposure in testis and placenta (Figure 3C). The *TCL1* gene expression, however, was detected in most hematopoietic tissues after several days exposure (Figure 3A). Semiquantitative RT-PCR analysis (Figure 4A) revealed that both *TCL1* and *TCL1b* genes are expressed in spleen, tonsil, fetal liver, fetal kidney, and fetal thymus. However, the *TCL1b* gene is expressed in wider variety of

tissues including placenta, kidney and fetal spleen (Figure 4A). Northern analysis of commercial human cancer cell lines showed that *TCL1* and *TCL1b* are expressed in only the Raji Burkitt lymphoma cell line (Figure 3B), although *TCL1* was expressed at a much higher level (Figure 3B).

5 The *TCL1* and *TCL1b* genes have similar transcription patterns and are physically linked. Therefore, a determination as whether the *TCL1b* gene could also be activated by rearrangements in 14q32 was made. Figures 3C and 3D show the activation of the *TCL1b* gene in a T-leukemia cell line with a translocation at 14q32.1 (SupT11) compared with the normal bone marrow and with EBV
10 transformed lymphoblastoid B cell lines expressing *TCL1*. (Figures 3C and 3D, middle panels). Since *TCL1* and *TCL1b* are normally not expressed in post-thymic T-cells and post-thymic T-cell leukemias lacking 14q32.1 abnormalities (for example, in T-ALL MOLT4 with no abnormalities at 14q32.1, Fig. 3B, lane 4), the expression of *TCL1* and *TCL1b* in SupT11 cells carrying a t(14;14)(q11;q32.1)
15 translocation indicates that juxtaposition of *TCL1* and *TCL1b* to the a/d locus of the T-cell receptor deregulates both genes.

To further investigate *TCL1b* expression, four T-cell leukemias and six EBV transformed lymphoblastoid cell lines with elevated levels of *TCL1* were analyzed. Figure 4B shows the activation of the *TCL1b* expression in one leukemic sample
20 from a patient with T-cell prolymphocytic leukemia. Human T-cell prolymphocytic leukemias carry the 14q32.1 translocation or inversion and overexpress *TCL1* (Virgilio, L., et al., 1994, *Proc Natl Acad Sci USA*, 91:12530-12534; Narducci, M.G., et al., 1997, *Cancer Res*, 57:5452-5456). The *TCL1b* gene was also expressed in two out of six EBV transformed lymphoblastoid B cell lines (Figure
25 3D, upper panel, lanes 2-7).

Discussion

The present invention discloses the cloning, mapping and expression analysis
30 of a novel member of the *TCL1* gene family, *TCL1b*. The *TCL1* and *TCL1b* genes are physically linked, show structural similarity, similar expression patterns and involvement in T-cell malignancies. Because the remaining two members of the

TCL1 family are oncogenes (Virgilio, L., et al., *Proc Natl Acad Sci USA*, 95:3885-3889; Gritti, C., et al., 1998, *Blood*, 92:368-373), it seems likely that *TCL1b* is also an oncogene. It is also likely that *TCL1b* activation would explain cases of T-cell leukemia with amplification at 14q32 without activation of *TCL1*.

It is possible that two *TCL1* genes are the result of duplication, although the *TCL1b* gene is slightly more homologous to the *MTCP1* gene at Xq28 than to the *TCL1* gene.

Neither the *in vivo* function of Tc11, nor the mechanism(s) of its oncogenic potential is known, although its crystal structure (Fu, Z.Q., et al., 1998, *Proc Natl Acad Sci USA*, 95:3413-3418) suggests, it may function as a transporter of small molecules, such as retinoids, nucleosides or fatty acids. The same study (Fu, Z.Q., et al., 1998, *Proc Natl Acad Sci USA*, 95:3413-3418) suggested that Tc11 might function as dimer, implying the possibility that Tc11 and Tc11b might form heterodimers.

Since *TCL1* and *MTCP1* transgenic mice develop mature T-cell leukemia only after 15 months (Virgilio, L., et al., 1998, *Proc Natl Acad Sci USA*, 95:3885-3889; Gritti, C., et al., 1998, *Blood*, 92:368-373), it will be of considerable interest to determine whether *TCL1b* transgenic mice also develop mature T-cell leukemia late and whether *TCL1* and *TCL1b* double transgenic mice develop leukemia faster. Thus, it seems possible that translocations and inversions at 14q32.1 contribute to malignant transformation by activating two oncogenes at the same time.

The present invention relates to nucleotide sequences of *TCL-1b* genes (SEQ. ID. NO: 11); and amino acid sequences of their encoded Tc1-1b proteins (SEQ. ID. NO: 10), as well as derivatives and analogs thereof, and antibodies thereto. The present invention further relates to the use of *TCL-1b* genes and their encoded proteins or derivatives or analogs thereof, and antibodies thereto, in assays for the detection and in treatment/prevention of disease states associated with chromosomal abnormalities and/or increased expression of *TCL1b*. The present invention also relates to therapeutic compositions comprising Tc1-1b proteins, derivatives or analogs thereof, antibodies thereto, nucleic acids encoding the Tc1-1b proteins, derivatives or analogs, and *TCL-1b* antisense nucleic acid.

The *Tcl-1b* gene sequence is from one of many different species, including but not limited to, mammalian, bovine, ovine, porcine, equine, rodent and human, in naturally occurring sequence or in variant form, or from any source, whether natural, synthetic, or recombinant. In a specific embodiment described herein, the

5 *Tcl-1b* gene sequence is a human sequence. The *Tcl-1b* protein is that present in one of many different species, including but not limited to, mammalian, bovine, ovine, porcine, equine, rodent and human, in naturally occurring or variant form, or from any source, whether natural, synthetic, or recombinant. In the specific embodiment described herein, the *Tcl-1b* protein is a human protein.

10 As defined herein, a *Tcl-1b* derivative is a fragment or amino acid variant of the *Tcl-1b* sequence (SEQ. ID. NO: 10);, as long as the fragment or amino acid variant is capable of displaying one or more biological activities associated with a full-length *Tcl-1b* protein. Such biological activities include, but are not limited to, antigenicity, i.e., the ability to bind to an anti-*Tcl-1b* antibody, and

15 immunogenicity, i.e., the ability to generate an antibody which is capable able of binding a *Tcl-1b* protein.

The invention provides fragments of a *Tcl-1b* protein consisting of at least 10 amino acids, or of at least 25 amino acids, or of at least 50 amino acids, or of at least 114 amino acids. Nucleic acids encoding such derivatives or analogs are also

20 within the scope of the invention. A preferred *Tcl-1b* protein variant is one sharing at least 70% amino acid sequence homology, a particularly preferred *Tcl-1b* protein variant is one sharing at least 80% amino acid sequence homology and another particularly preferred *Tcl-1b* protein variant is one sharing at least 90% amino acid sequence homology to the naturally occurring *Tcl-1b* protein over at least 25, at least

25 50, at least 75 or at least 100 contiguous amino acids of the *Tcl-1b* amino acid sequence. As used herein, amino acid sequence homology refers to amino acid sequences having identical amino acid residues or amino acid sequences containing conservative changes in amino acid residues. In another embodiment, a *Tcl-1b* homologous protein is one that shares the foregoing percentages of sequences

30 identical with the naturally occurring *Tcl-1b* protein over the cited lengths of amino acids.

The *TCL-1b* gene (SEQ. ID. NO: 11); is located in the region of chromosome 14q32.1 that is located in a region banded by two clusters of breakpoints. Due to the similarities between the *TCL1* and *TCL-1b* gene structure, sequence and location, their expression patterns were compared. Expression in
5 normal tissue was mostly negative for *TCL1b*, Figure 3A. The *TCL1* gene expression, however, was detected in most hematopoietic tissues and both *TCL1* and *TCL1b* are expressed in spleen, tonsil, fetal liver, fetal kidney and fetal thymus. The *TCL1b* gene is expressed in a wider variety of tissues including placenta, kidney and fetal spleen, as shown in Figure 4A. The detection of *TCL-1b* mRNA in patient
10 samples, such as biopsied cells and tissues, is used as an indicator of the presence of T-cell leukemias and lymphomas associated with certain chromosome 14 abnormalities and/or increased expression of Tcl-1b protein. Also, the Tcl -1b amino acid sequences of the present invention are used to generate antibodies useful in immunoassays for the detection or measurement of Tcl -1b in patient samples.
15 Such anti- Tcl -1b antibodies are used in diagnostic immunoassays, for the detection or measurement of increased levels of Tcl -1b associated with T-cell leukemias and lymphomas.

In accordance with the present invention, polynucleotide sequences coding for a Tcl-1b protein (SEQ. ID. NO: 10);, derivative, e.g. fragment, or analog
20 thereof, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence, for the generation of recombinant DNA molecules that direct the expression of a Tcl-1b protein. Such Tcl -1b polynucleotide sequences, as well as other polynucleotides or their complements, are also used in nucleic acid
25 hybridization assays, Southern and Northern blot analysis, etc. In a specific embodiment, a human *TCL-1b* gene (SEQ. ID. NO: 11);, or a sequence encoding a functionally active portion of a human *TCL-1b* gene, is expressed. In yet another embodiment, a derivative or fragment of a human *TCL-1b* gene is expressed.

30 The TCL-1b Coding Sequences

In a specific embodiment disclosed herein by reference to the expressed sequence tag (EST) database accession no. AA689513, the invention relates to the

nucleic acid sequence of the human *TCL-1b* gene (SEQ. ID. NO: 11). In a preferred, but not limiting, aspect of the invention, a human *TCL-1b* cDNA sequence (SEQ. ID. NO: 9); was identified in the expressed sequence tag database (accession no. AA689513) that was homologous to *TCL-1* and *MTCPI*, the other
5 members of the *TCL1* gene family. Such a sequence was isolated and cloned as a 1.2 kilobase full-length cDNA, as described, *infra*. The invention also relates to nucleic acid sequences hybridizable or complementary to the foregoing sequences, or equivalent to the foregoing sequences, in that the equivalent nucleic acid sequences also encode a Tcl-1b protein product.

10 In a preferred aspect, polymerase chain reaction (PCR) is used to amplify the desired nucleic acid sequence in the library by using oligonucleotide primers representing known *TCL-1b* sequences (SEQ. ID. NO: 9). Such primers are used to amplify sequences of interest from an RNA or DNA source, preferably a cDNA library. PCR is carried out by use of a Perkin-Elmer Cetus thermal cycler and Taq
15 polymerase, as is well known by those skilled in the art. The DNA being amplified is mRNA or cDNA or genomic DNA from any eukaryotic species. Several different degenerate primers are synthesized for use in PCR amplification reactions. The stringency of hybridization conditions used in priming the PCR reactions are also varied in order to allow for greater or lesser degrees of nucleotide sequence
20 homology between the *TCL-1b* gene being cloned and that of the *TCL-1b* gene (SEQ. ID. NO: 11) of the present invention.

After successful amplification of a segment of the *TCL-1b* gene, an allelic, a polymorphic variant, or a species homology of the *TCL-1b* gene, that segment is molecularly cloned and sequenced, and utilized as a probe to isolate a complete
25 cDNA or genomic clone. This will permit the determination of the gene's complete nucleotide sequence, the analysis of its expression, and the production of its protein product for functional analysis. This allows for the identification of additional genes encoding the Tcl-1b proteins.

Potentially, any eukaryotic cell can serve as the nucleic acid source for the
30 molecular cloning of the *TCL-1b* gene. The nucleic acid sequences encoding *TCL-1b* are isolated from, for example, human, porcine, bovine, feline, avian, equine, canine, rodent, as well as additional primate sources. The DNA is obtained by

standard procedures known in the art from, for example, cloned DNA (e.g., a DNA "library"), by chemical synthesis, by cDNA cloning, or by the cloning of genomic DNA, or fragments thereof, purified from a desired cell. (See, for example, Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; Glover, D. M. (ed.), 1985, *DNA Cloning: A Practical Approach*, MRL Press, Ltd., Oxford, U.K. Vol. I, II.) A preferred source is cDNA of leukemic cells in which the leukemia is associated with a 14q32.1 chromosomal abnormality. Clones derived from genomic DNA contain regulatory and intron DNA regions in addition to coding regions, while clones derived from cDNA will contain only *TCL-1b* exon sequences. In a particular embodiment of the present invention, a genomic sequence is one that is not more than 10 kilobases (kb), or not more than 20 kb, or not more than 50 kb or not more than 70 kb. Whatever the source, the gene should be molecularly cloned into a suitable vector for propagation of the gene. In a particular embodiment, a preferred source of nucleic acid for the isolation of *TCL-1b* gene sequences is from pre B-cells.

In the molecular cloning of the gene from genomic DNA, DNA fragments are generated, some of which will encode the desired gene. The DNA is cleaved at specific sites using various restriction enzymes. Alternatively, DNase in the presence of manganese is used to fragment the DNA, or the DNA is physically sheared, as for example, by sonication. The linear DNA fragments is then separated according to size by standard techniques, including but not limited to, agarose and polyacrylamide gel electrophoresis and column chromatography.

Once the DNA fragments are generated, identification of the specific DNA fragment containing the desired gene is accomplished in a number of ways. For example, a *TCL-1b* gene (SEQ. ID. NO: 11) of the present invention or its specific RNA, or a fragment thereof, such as a probe or primer, is isolated and labeled and then used in hybridization assays to detect a generated *TCL-1b* gene (Benton, W. and Davis, R., 1977, *Science*, 196:180; Grunstein, M. and Hogness, D., 1975, *Proc Natl Acad Sci USA*, 72:3961). Those DNA fragments sharing substantial sequence homology to the probe will hybridize under stringent conditions. The phrase "stringent conditions" as used herein refers to those hybridizing conditions

that (Virgilio, L., et al., 1994, *Proc Natl Acad Sci USA*, 91:12530-12534) employ low ionic strength and high temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50.degree. C.; (Narducci, M.G., et al., 1997, *Cancer Res*, 57:5452-5456) employ, during hybridization, a denaturing agent such as formamide, for example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42.degree. C.; or (3) employ 50% formamide, 5.times.SSC (0.75 M NaCl, 0.075 M sodium pyrophosphate, 5.times.Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42.degree. C., with washes at 42.degree. C. in 0.2.times.SSC and 0.1% SDS.

The appropriate fragment is also identified by restriction enzyme digestion(s) and comparison of fragment sizes with those expected according to a known restriction map. Further selection is carried out on the basis of the properties of the gene. Alternatively, the presence of the gene is detected by assays based on the physical, chemical, or immunological properties of its expressed product. For example, cDNA clones, or genomic DNA clones which hybrid-select the proper mRNAs, are selected which produce a protein that has similar or identical electrophoretic migration, isoelectric focusing behavior, proteolytic digestion maps, binding activity or antigenic properties as known for Tcl-1b. Alternatively, the Tcl-1b protein may be identified by binding of labeled antibody to the putatively Tcl-1b expressing clones, e.g., in an ELISA (enzyme-linked immunosorbent assay)-type procedure.

The *TCL-1b* gene is also identified by mRNA selection by nucleic acid hybridization followed by *in vitro* translation. In this procedure, fragments are used to isolate complementary mRNAs by hybridization. Such DNA fragments may represent available, purified *TCL-1b* DNA of another *TCL-1b* gene. Immunoprecipitation analysis, or functional assays, of the *in vitro* translation products of the isolated products of the isolated mRNAs identifies the mRNA and, therefore, the complementary DNA fragments that contain the desired sequences. In addition, specific mRNAs may be selected by adsorption of polysomes isolated from cells to immobilized antibodies specifically directed against Tcl-1b protein. A

radiolabelled *TCL-1b* cDNA is synthesized using the selected mRNA (from the adsorbed polysomes) as a template. The radiolabelled mRNA or cDNA is then used as a probe to identify the *TCL-1b* DNA fragments from among other genomic DNA fragments.

- 5 Alternatives to isolating the *TCL-1b* genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the Tcl-1b protein. For example, RNA useful in cDNA cloning of the *TCL-1b* gene is isolated from cells which express Tcl-1b, e.g., pre-B acute lymphoblastic leukemia cells or endemic Burkitt's
- 10 lymphoma cells which express cell surface IgM and do not secrete immunoglobulin. Other methods are known to those of skill in the art and are within the scope of the invention.

- The identified and isolated gene is then inserted into an appropriate cloning vector. A large number of vector-host systems known in the art may be used.
- 15 Possible vectors include, but are not limited to, plasmids or modified viruses, but the vector system must be compatible with the host cell used. Such vectors include, but are not limited to, bacteriophages such as lambda derivatives, or plasmids such as PBR322 or pUC plasmid derivatives. The insertion into a cloning vector can, for example, be accomplished by ligating the DNA fragment into a cloning vector
- 20 which has complementary cohesive termini. However, if the complementary restriction sites used to fragment the DNA are not present in the cloning vector, the ends of the DNA molecules may be enzymatically modified. Alternatively, any site desired may be produced by ligating nucleotide sequences (linkers) onto the DNA termini; these ligated linkers comprise specific chemically synthesized
- 25 oligonucleotides encoding restriction endonuclease recognition sequences. In an alternative method, the cleaved vector and *TCL-1b* gene is modified by homopolymeric tailing. Recombinant molecules are introduced into host cells via transformation, transfection, infection, electroporation, or other methods known to those of skill in the art, so that many copies of the gene sequence are generated.
- 30 In an alternative method, the desired gene is identified and isolated after insertion into a suitable cloning vector in a "shot gun" approach. Enrichment for the

desired gene, for example, by size fractionization, is done before insertion into the cloning vector.

In specific embodiments, transformation of host cells with recombinant DNA molecules that incorporate the isolated *TCL-1b* gene, cDNA, or synthesized DNA sequence enables generation of multiple copies of the gene. Thus, the gene is obtained in large quantities by growing transformants, isolating the recombinant DNA molecules from the transformants and, when necessary, retrieving the inserted gene from the isolated recombinant DNA.

Oligonucleotides containing a portion of the *TCL-1b* coding or non-coding sequences, or which encode a portion of the Tcl-1b protein (e.g., primers for use in PCR) are synthesized by standard methods commonly known in the art. Such oligonucleotides preferably have a size in the range of 8 to 25 nucleotides. In a particular embodiment herein, such oligonucleotides have a size in the range of 15 to 25 nucleotides or 18 to 25 nucleotides.

Expression of the *TCL-1b* Gene

In accordance with the present invention, polynucleotide sequences coding for a Tcl-1b protein, derivative, e.g. fragment, or analog thereof, can be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for the transcription and translation of the inserted protein-coding sequence, for the generation of recombinant DNA molecules that direct the expression of a Tcl-1b protein. Such *TCL-1b* polynucleotide sequences, as well as other polynucleotides or their complements, may also be used in nucleic acid hybridization assays, Southern and Northern blot analysis, etc. In a specific embodiment, a human *TCL-1b* gene, or a sequence encoding a functionally active portion of a human *TCL-1b* gene is expressed. In yet another embodiment, a derivative or fragment of a human *TCL-1b* gene is expressed.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent Tcl-1b amino acid sequence, is within the scope of the invention. Such DNA sequences include those

which are capable of hybridizing to the human *TCL-1b* sequence under stringent conditions.

Altered DNA sequences which are used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within a *TCL-1b* sequence, which result in a silent change, thus producing a functionally equivalent Tcl-1b protein. Such amino acid substitutions are made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: leucine, isoleucine, valine; glycine, alanine; asparagine, glutamine; serine, threonine; phenylalanine, tyrosine.

The DNA sequences of the invention are engineered in order to alter a *TCL-1b* coding sequence for a variety of ends, including but not limited to alterations which modify processing and expression of the gene product. For example, mutations introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter phosphorylation, etc.

In another embodiment of the invention, a *TCL-1b* gene sequence or a derivative thereof is ligated to a non-*TCL-1b* sequence to encode a chimeric fusion protein. A fusion protein is engineered to contain a cleavage site located between a Tcl-1b sequence and the non-Tcl-1b protein sequence, so that the Tcl-1b protein may be cleaved away from the non-Tcl-1b moiety. In a specific embodiment, the Tcl-1b amino acid sequence present in the fusion protein consists of at least 10 contiguous amino acids, at least 25 contiguous amino acids, at least 50 contiguous amino acids, at least 75 contiguous amino acids, at least 100 contiguous amino acids, or at least 114 amino acids of the Tcl-1b protein sequence.

In an alternate embodiment of the invention, the coding sequence of a Tcl-1b is synthesized in whole or in part, using chemical methods well known in the art. See, for example, Caruthers et al., 1980, *Nuc Acids Res Symp Ser*, 7:215-233; Crea

and Horn, 1980, *Nuc Acids Res*, 9(10):2331; Matteucci and Caruthers, 1980, *Tetrahedron Letters* 21:719; and Chow and Kempe, 1981, *Nuc Acids Res* 9(12):2807-2817. Alternatively, the protein itself is produced using chemical methods to synthesize a Tcl -1b amino acid sequence in whole or in part. For
5 example, peptides are synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, *Proteins Structures And Molecular Principles*, W. H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see
10 Creighton, 1983, *Proteins, Structures and Molecular Principles*, W. H. Freeman and Co., N.Y., pp. 34-49.

In order to express a biologically active Tcl -1b protein or derivative thereof, a polynucleotide sequence encoding a Tcl-1b protein, or a derivative thereof, is inserted into an appropriate expression vector, i.e., a vector which contains the
15 necessary elements for the transcription and translation of the inserted coding sequence. The *TCL-1b* gene products, as well as host cells or cell lines transfected or transformed with recombinant *TCL-1b* expression vectors, are used for a variety of purposes. These include, but are not limited to, generating antibodies (i.e., monoclonal or polyclonal) that immunospecifically bind a Tcl -1b protein. Anti- Tcl
20 -1b antibodies are used in detecting or measuring levels of a Tcl -1b protein in patient samples.

Expression Systems

25 Methods which are well known to those skilled in the art are used to construct expression vectors containing a *TCL-1b* coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in
30 Sambrook et al., 1989, *Molecular Cloning, A Laboratory Manual* 2d ed., Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y.

A variety of host-expression vector systems are utilized to express a *TCL-1b* coding sequence. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing an *TCL-1b* coding sequence; yeast transformed with recombinant yeast expression vectors containing an *TCL-1b* coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing an *TCL-1b* coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing a *TCL-1b* coding sequence; or animal cell systems. The expression elements of these systems vary in their strength and specificities. Depending on the host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, are used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage.lambda., plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like are used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter are used; when cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll a/b binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) are used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5 K promoter) are used; when generating cell lines that contain multiple copies of an *TCL-1b* DNA, SV40-, BPV- and EBV-based vectors are used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors are advantageously selected depending upon the use intended for the *Tcl-1b* protein expressed. For example, when large quantities of *Tcl-1b* protein are produced for the generation of antibodies, vectors which direct the expression of high levels of fusion protein products that are readily purified are desirable. Such vectors include, but are not

limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J*, 2:1791), in which the *TCL-1b* coding sequence are ligated into the vector in frame with the lac Z coding region so that a hybrid AS-lac Z protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res*, 13:3101-3109; Van Heeke & Schuster, 1989, *J Biol Chem*, 264:5503-5509); and the like. pGEX vectors are also
5 used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and easily purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The PGEX vectors are designed to include
10 thrombin or factor Xa protease cleavage sites so that the cloned polypeptide of interest is released from the GST moiety.

In yeast, a number of vectors containing constitutive or inducible promoters are used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Ed. Wu & Grossman, 1987, Acad. Press, N.Y. 153:516-544; Glover, 1986, DNA Cloning. Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y. 152:673-684; and The Molecular Biology of the Yeast *Saccharomyces*,
20 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II.

In cases where plant expression vectors are used, the expression of a *TCL-1b* coding sequence is driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, *Nature* 310:511-514), or the coat protein promoter of TMV (Takamatsu et al., 1987, *EMBO J*, 6:307-311) are used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, *EMBO J*, 3:1671-1680; Broglie et al., 1984, *Science*, 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley et al., 1986, *Mol Cell. Biol*, 6:559-565) are used. These constructs are introduced into plant cells using Ti plasmids, Ri plasmids, plant virus
30 vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, N.Y., Section VIII, pp.

421-463; and Grierson & Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express a *TCL-1b* gene is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. A *TCL-1b* coding sequence is cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example, the polyhedrin promoter). Successful insertion of a *TCL-1b* coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect *Spodoptera frugiperda* cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, *J Virol*, 46:584; Smith, U.S. Pat. No. 4,215,051).

In mammalian host cells, a number of viral based expression systems are utilized. In cases where an adenovirus is used as an expression vector, a *TCL-1b* coding sequence is ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene is then inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a *TCL-1b* in infected hosts. (e.g., see Logan & Shenk, 1984, *Proc Natl Acad Sci USA*, 81:3655-3659). Alternatively, the vaccinia 7.5 K promoter are used. (See, e.g., Mackett et al., 1982, *Proc Natl Acad Sci USA*, 79:7415-7419; Mackett et al., 1984, *J Virol*, 49:857-864; Panicali et al., 1982, *Proc Natl Acad Sci USA*, 79:4927-4931).

Specific initiation signals may also be required for efficient translation of an inserted *TCL-1b* coding sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire *TCL-1b* gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only a portion of a *TCL-1b* coding sequence is inserted, lacking the 5' end, exogenous translational control signals, including the ATG initiation codon, must be

provided. Furthermore, the initiation codon must be in phase with the reading frame of a *TCL-1b* coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons are of a variety of origins, both natural and synthetic. The efficiency of expression are enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol*, 153:516-544).

In addition, a host cell strain is chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., phosphorylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cells lines or host systems are chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, and phosphorylation of the gene product are used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express a Tc1 -1b protein are engineered. Rather than using expression vectors which contain viral origins of replication, host cells are transformed with *TCL-1b* DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker.

Following the introduction of foreign DNA, engineered cells are allowed to grow for 1-2 days in an enriched media, and are then switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn are cloned and expanded into cell lines. This method is advantageously used to engineer cell lines which express a Tc1-1b protein. The present invention provides a method for producing a recombinant Tc1 -1b protein comprising culturing a host cell transformed with a recombinant expression vector

encoding a TcI -1b protein such that the TcI -1b protein is expressed by the cell and recovering the expressed TcI -1b protein.

A number of selection systems are used, including, but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell*, 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, *Proc Natl Acad Sci USA*, 48:2026), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell*, 22:817) genes can be employed in tk-, hgp^rt- or ap^rt-cells, respectively. Also, antimetabolite resistance is used as the basis of selection for dhfr, which confers resistance to methotrexate (Wigler et al., 1980, *Natl Acad Sci USA*, 77:3567; O'Hare et al., 1981, *Proc Natl Acad Sci USA*, 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc Natl Acad Sci USA*, 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., 1981, *J Mol Biol*, 150:1); and hyg^r, which confers resistance to hygromycin (Santerre et al., 1984, *Gene*, 30:147). Recently, additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, *Proc Natl Acad Sci USA*, 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, L., 1987, In: *Current Communications in Molecular Biology*, Cold Spring Harbor Laboratory, Ed.).

Identification of Transfectants or Transformants That Express TcI-1b

The host cells which contain the coding sequence and which express the biologically active gene product are identified by at least four general approaches; (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of transcription as measured by the expression of TCL-1b mRNA transcripts in the host cell; and (d) detection of the gene product as measured by immunoassay or by its biological activity.

In the first approach, the presence of the *TCL-1b* coding sequence inserted in the expression vector is detected by DNA-DNA or DNA-RNA hybridization using

probes comprising nucleotide sequences that are homologous to the *TCL-1b* coding sequence, respectively, or portions or derivatives thereof.

In the second approach, the recombinant expression vector/host system is identified and selected based upon the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the human *TCL-1b* coding sequence is inserted within a marker gene sequence of the vector, recombinant cells containing the *TCL-1b* coding sequence is identified by the absence of the marker gene function. Alternatively, a marker gene is placed in tandem with a *TCL-1b* sequence under the control of the same or different promoter used to control the expression of the *TCL-1b* coding sequence. Expression of the marker in response to induction or selection indicates expression of the *TCL-1b* coding sequence.

In the third approach, transcriptional activity of a *TCL-1b* gene is assessed by hybridization assays. For example, RNA is isolated and analyzed by Northern blot using a probe having sequence homology to a *TCL-1b* coding sequence or transcribed noncoding sequence or particular portions thereof. Alternatively, total nucleic acid of the host cell are extracted and quantitatively assayed for hybridization to such probes.

In the fourth approach, the levels of a Tcl-1b protein product is assessed immunologically, for example by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

Purification of the Expressed Gene Product

Once a recombinant which expresses the *TCL-1b* gene sequence is identified, the gene product is analyzed. This is achieved by assays based on the physical or functional properties of the product, including radioactive labelling of the product followed by analysis by gel electrophoresis, immunoassay, or other detection methods known to those of skill in the art.

Once the Tcl-1b protein is identified, it is isolated and purified by standard methods including chromatography (e.g., ion exchange, affinity, and sizing column

chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. The functional properties are evaluated using any suitable assay.

Alternatively, once a TcI-1b protein produced by a recombinant is identified, the amino acid sequence of the protein is deduced from the nucleotide sequence of the chimeric gene contained in the recombinant. As a result, the protein is synthesized by standard chemical methods known in the art (e.g., see Hunkapiller et al., 1984, *Nature*, 310:105-111).

In a specific embodiment of the present invention, such TcI -1b proteins, whether produced by recombinant DNA techniques or by chemical synthetic methods, include, but are not limited to, those containing, as a primary amino acid sequence, all or part of the amino acid sequence substantially as depicted in Figure 1 (SEQ ID NO:10), as well as fragments and other derivatives, and analogs thereof.

15 Generation of Antibodies to TcI-1b

According to the invention, TcI-1b protein, its fragments or other derivatives, or analogs thereof, are used as an immunogen to generate antibodies which recognize such an immunogen. Such antibodies include but are not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments, and an Fab expression library. In a specific embodiment, antibodies to a human TcI -1b protein are produced.

Various procedures known in the art are used for the production of polyclonal antibodies to a TcI -1b protein or derivative or analog. For the production of antibody, various host animals are immunized by injection with the native TcI -1b protein, or a synthetic version, or derivative (e.g., fragment) thereof, including but not limited to rabbits, mice, rats, etc. Various adjuvants are used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins,

dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum.

In a specific example, the 14 kDa protein of the TCL-1b gene expressed in bacteria was used to immunize rabbits against Tcl -1b. Such antibodies recognized
5 the 14 kDa Tcl -1b protein in a variety of leukemia and lymphoma cells by Western Blot and by immunoprecipitation.

For preparation of monoclonal antibodies directed toward a Tcl -1b protein sequence (SEQ. ID. NO: 10) or analog thereof, any technique which provides for the production of antibody molecules by continuous cell lines in culture are used.
10 For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature*, 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today*, 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In an
15 additional embodiment of the invention, monoclonal antibodies are produced in germ-free animals utilizing recent technology (PCT/US90/02545). According to the invention, human antibodies are used and are obtained by using human hybridomas (Cote et al., 1983, *Proc Natl Acad Sci USA*, 80:2026-2030) or by transforming human B cells with EBV virus in vitro (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, pp. 77-96). In fact, according to the invention,
20 techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc Natl Acad Sci USA*, 81:6851-6855; Neuberger et al., 1984, *Nature*, 312:604-608; Takeda et al., 1985, *Nature*, 314:452-454) by splicing the genes from a mouse antibody molecule specific for TCL-1b together with genes from a human
25 antibody molecule of appropriate biological activity is used; such antibodies are within the scope of this invention.

According to the invention, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) are adapted to produce Tcl -1b-specific single chain antibodies. An additional embodiment of the invention utilizes the
30 techniques described for the construction of Fab expression libraries (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal

Fab fragments with the desired specificity for Tc1-1b proteins, derivatives, or analogs.

Antibody fragments which contain the idiotype of the molecule are generated by known techniques. For example, such fragments include, but are not limited to, the F(ab')₂ fragment which is produced by pepsin digestion of the antibody molecule; the Fab' fragments which are generated by reducing the disulfide bridges of the F(ab')₂ fragment, and the Fab fragments which are generated by treating the antibody molecule with papain and a reducing agent.

In the production of antibodies, screening for the desired antibody is accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of a Tc1-1b protein, one may assay generated hybridomas for a product which binds to a Tc1-1b fragment containing such domain. For selection of an antibody specific to human Tc1-1b, one can select on the basis of positive binding to human Tc1-1b and a lack of binding to, for example, mouse Tc1-1b.

The foregoing antibodies are used in methods known in the art relating to the localization and activity of the protein sequences of the invention, e.g., for imaging these proteins, measuring levels thereof in appropriate physiological samples, etc.

20 Structure of the Tc1-1b Gene and Protein

The structure of the Tc1-1b gene and protein is analyzed by various methods known in the art.

25 Genetic Analysis

The cloned DNA or cDNA corresponding to the *TCL-1b* gene is analyzed by methods including but not limited to Southern hybridization (Southern, E. M., 1975, *J Mol Biol*, 98:503-517), Northern hybridization (see, e.g., Freeman et al., 1983, *Proc Natl Acad Sci USA*, 80:4094-4098), restriction endonuclease mapping (Maniatis, T., 1982, *Molecular Cloning, A Laboratory*, Cold Spring Harbor, N.Y.), and DNA sequence analysis. Polymerase chain reaction (PCR; U.S. Pat.

Nos. 4,683,202, 4,683,195, and 4,889,818; *Proc Natl Acad Sci USA*, 85:7652-7656; Ochman et al., 1988, *Genetics*, 120:621-623; Loh et al., 1989, *Science*, 243:217-220) followed by Southern hybridization with a *TCL-1b*-specific probe allows the detection of the *TCL-1b* gene in DNA from various cell types. In one embodiment, Southern hybridization is used to determine the genetic linkage of *TCL-1b*. PCR followed by hybridization assay is also used to detect or measure *TCL-1b* RNA or 14q32.1 chromosomal abnormalities. Northern hybridization analysis is used to determine the expression levels of the *TCL-1b* gene. Various cell types, at various states of development or activity are tested for *TCL-1b* expression.

10 The stringency of the hybridization conditions for both Southern and Northern hybridization, or dot blots, are manipulated to ensure detection of nucleic acids with the desired degree of relatedness to the specific *TCL-1b* probe used.

Restriction endonuclease mapping is used to roughly determine the genetic structure of the *TCL-1b* gene. Restriction maps derived by restriction endonuclease cleavage are confirmed by DNA sequence analysis.

DNA sequence analysis is performed by any techniques known in the art, including, but not limited to, the method of Maxam and Gilbert (1980, *Meth Enzymol*, 65:499-560), the Sanger dideoxy method (Sanger et al., 1977, *Proc Natl Acad Sci USA*, 74:5463), the use of T7 DNA polymerase (Tabor and Richardson, U.S. Pat. No. 4,795,699), or use of an automated DNA sequenator (e.g., Applied Biosystems, Foster City, Calif.). The cDNA sequence of a representative *TCL-1b* gene comprises the sequence substantially as disclosed herein (SEQ. ID. NO: 9).

Protein Analysis

25

The amino acid sequence of the *Tcl-1b* protein is derived by deduction from the DNA sequence, or alternatively, by direct sequencing of the protein, e.g., with an automated amino acid sequencer. The amino acid sequence of a representative *Tcl-1b* protein comprises the sequence substantially as depicted in Figure 1 (SEQ ID NO: 10), with the representative mature protein that is shown by amino acid numbers 1-128.

30

The TcI -1b protein sequence is further characterized by a hydrophilicity analysis (Hopp, T. and Woods, K., 1981, *Proc Natl Acad Sci USA*, 78:3824). A hydrophilicity profile is used to identify the hydrophobic and hydrophilic regions of the TcI -1b protein and the corresponding regions of the gene sequence which
5 encode such regions.

Secondary structural analysis (Chou, P. and Fasman, G., 1974, *Biochemistry*, 13:222) is also done, to identify regions of the TcI-1b protein that assume specific secondary structures.

Manipulation, translation, and secondary structure prediction, as well as
10 open reading frame prediction and plotting, is also accomplished using computer software programs available in the art.

Other methods of structural analysis are also employed. These include, but are not limited to, X-ray crystallography (Engstrom, A., 1974, *Biochem Exp Biol* 11:7-13) and computer modeling (Fletterick, R. and Zoller, M. (eds.), 1986,
15 Computer Graphics and Molecular Modeling, in Current Communications in Molecular Biology, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

Uses of TCL-1b and its TcI-1b Protein Product and Antibodies Thereto

20 Chromosomal translocations and inversions associated with the *TCL-1b* locus on chromosome 14, e.g., t(14:14)(q11;q32) chromosome translocation, inv(14)(q11;q32) chromosome inversion, and t(7:14)(q35;q32) chromosome translocation, are associated with several post-thymic types of T-cell leukemias, including, but not limited to, T-prolymphocytic leukemias (T-PLL) (Brito-Babapulle
25 and Catovsky, 1991, *Cancer Genet Cytogenet*, 55:1-9), acute and chronic leukemias associated with the immunodeficiency syndrome ataxia-telangiectasia (AT) (Russo et al., 1988, *Cell*, 53:137-144; Russo et al., 1989, *Proc Natl Acad Sci USA*, 86:602-606), and adult T-cell leukemia (Virgilio et al., 1993, *PNAS*, 90:9275-9279). In some cases of AT-associated translocations, in T-cell leukemia and lymphoma
30 involving the 14q32.1 band, clonal expansion of cells carrying abnormalities in 14q32.1 have been documented in some cases prior to the development of overt malignancy (Russo, et al., 1988, *Cell*, 53:137-144). Therefore, a TCL-1b

polynucleotide, its Tcl -1b protein product and antibodies thereto is used for diagnostic and/or therapeutic/prophylactic purposes for the above described diseases, as well as other disorders associated with chromosomal translocations and inversions associated with the TCL-1b locus and/or, increased expression of TCL-1b RNA or protein. A TCL-1b polynucleotide, its Tcl -1b protein product and antibodies thereto are used for therapeutic/prophylactic purposes alone or in combination with other therapeutics useful in the treatment of T-cell leukemias. Such molecules are also used in diagnostic assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders associated with *TCL-1b* gene expression or monitor the treatment thereof. Accordingly, in specific embodiments, T-cell malignancies or premalignant changes in such tissues is diagnosed by detecting increased *TCL-1b* expression in patient samples relative to the level of *TCL-1b* expression in an analogous non-malignant sample (from the patient or another person, as determined experimentally or as is known as a standard level in such samples). For diagnostic purposes, a TCL-1b polynucleotide is used to detect *TCL-1b* gene expression or increased *TCL-1b* gene expression in disease states, such as, T-cell leukemias and lymphomas. For therapeutic purposes, a Tcl -1b protein is used to make anti- Tcl 1b antibodies that neutralize the activity of Tcl -1b. Included within the scope of the present invention are oligonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes, that function to inhibit expression of a TCL-1b RNA or protein.

Diagnostic Uses

As illustrated *infra*, the *TCL-1b* gene sequence is associated with disease states associated with chromosome 14 translocations and inversions around the *TCL-1b* locus, is preferentially expressed early in T and B lymphocyte differentiation and demonstrates a high level of expression in cells from patients diagnosed with T-PLL carrying an inversion of chromosome 14, *inv*(14)(q11;q32) or patients carrying a *t*(14:14)(q11;q32) chromosome translocation. Accordingly, the *TCL-1b* gene sequence (SEQ. ID. NO: 11) is used diagnostically for the detection of disease states resulting from chromosomal abnormalities, e.g., translocations, inversions

and deletions, involving the *TCL-1b* locus of chromosome 14. Nucleic acids comprising *TCL-1b* nucleotide sequences of at least 8 nucleotides, at least 15 nucleotides, at least 25 nucleotides, at least 50 nucleotides, at least 100 nucleotides, at least 200 nucleotides, at least 300 nucleotides, or at least 387 nucleotides up to 5 1324 nucleotides of SEQ ID NO: 9 are used as probes in hybridization assays for the detection and measurement of *TCL-1b* gene (SEQ. ID. NO: 11). Nucleic acids of not more than 5 kilobases, of not more than 10 kilobases, not more than 25 kilobases, not more than 50 kilobases or not more than 70 kilobases which are hybridizable to a *TCL-1b* gene, cDNA, or complementary strand is used as probes 10 in hybridization assays for the detection and measurement of *TCL-1b* nucleotide sequences. As an example, the *TCL-1b* DNA sequence is used in hybridization assays, e.g., Southern or Northern analysis, including in situ hybridization assays, of patient's samples to diagnose abnormalities of *TCL-1b* expression. Hybridization assays are used to detect, prognose, diagnose, or monitor conditions, disorders, or 15 disease states, such as T-cell malignancies, associated with aberrant changes in *TCL-1b* expression and/or activity as described supra. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to *TCL-1b* DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any 20 resulting hybridization. In particular, hybridization assays are used to detect the presence of abnormalities associated with increased expression of *TCL-1b* mRNA, by hybridizing mRNA or cDNA from a patient sample to a *TCL-1b* probe, and measuring the amount of resulting hybridization. For example, assays which are used include, but are not limited to Northern blots, Dot blots, reverse transcriptase 25 PCR, etc. A preferred hybridization assay is Northern blot analysis of a patient sample using *TCL-1b* gene probes of at least 15 polynucleotides up to the full length cDNA sequence (SEQ. ID. NO: 9) shown in Figure X. . Another preferred hybridization assay is in situ hybridization analysis of a patient sample using anti-Tcl-1b antibodies or *TCL-1b* nucleotide hybridization probes. Such techniques are 30 well known in the art, and are in fact the basis of many commercially available diagnostic kits.

As used herein, patient samples which are used include, but are not limited to, fresh or frozen tissue samples, which are used in in situ hybridization assays; cell or tissue samples containing T-lymphocytes and, in general, patient samples containing nucleic acid, such as peripheral blood lymphocytes (PBL) and T-lymphocytes which are used in assays that measure or quantitate *TCL-1b* nucleic acid.

Polynucleotide sequences of *TCL-1b* consisting of at least 8 to 25 nucleotides that are useful as primers in primer dependent nucleic acid amplification methods are used for the detection of *TCL-1b* gene sequences in patient samples. Primer dependent nucleic acid amplification methods useful in the present invention include, but are not limited to, polymerase chain reaction (PCR), competitive PCR, cyclic probe reaction, and ligase chain reaction. Such techniques are well known by those of skill in the art. A preferred nucleic acid amplification method of the present invention is reverse transcriptase PCR (RT-PCR) (Siebert et al., 1992, *Nature*, 359:557-558).

In a particular embodiment of the present invention, each primer of a pair of primers for use in a primer dependent nucleic acid amplification method is selected from a different exon of the genomic *TCL-1b* nucleotide sequences. For example, if one primer of a pair or primers is selected from exon 1 of the *TCL-1b* genomic sequence, the second primer will be selected from exon 2, 3 or 4 of the *TCL-1b* genomic sequence. As another example, if one primer of a pair of primers is selected from exon 2 of the *TCL-1b* genomic sequence, the second primer will be selected from exon 1, 3, or 4 of the *TCL-1b* genomic sequence. By selecting each primer of a pair of primers for use in a primer dependent nucleic acid amplification method from a different exon, amplified genomic nucleotide sequences are distinguished from amplified cDNA nucleotide sequences due to the size difference of the resulting amplified sequences. Resulting amplified genomic nucleotide sequences will contain amplified intron sequences and will be of a larger size than amplified cDNA nucleotide sequences that will not contain amplified intron sequences. For amplification of cDNA nucleotide sequences, the primer sequences should be selected from exons sequences that are sufficiently far enough apart to provide a detectable amplified nucleotide sequence.

The *TCL-1b* gene sequences (SEQ. ID. NO: 9 and 11) of the present invention are used diagnostically for the detection of chromosome 14 abnormalities, in particular translocations t(14:14)(q11;q32) and inv(14)(q11;q32) inversion at 14q32.1. Accordingly, the present invention provides a process for detecting a target sequence indicative of or including a chromosome 14 abnormality in a sample, comprising the steps of amplifying the target sequence in the sample using a first primer of 8 to 25 nucleotides, preferably 18-25 nucleotides, complementary to the nucleotide sequence of SEQ ID NO: 11 or SEQ ID NO: 9, and a second primer complementary to a region telomeric or centromeric to the *TCL-1b* gene and detecting any resulting amplified target sequence in which the presence of the amplified target sequence is indicative of the abnormality. The present invention also provides a method of diagnosing a T-cell malignancy associated with chromosome 14 abnormalities in a patient by detecting a chromosome 14 abnormality according to the method above in which the presence of the amplified target sequence indicates the presence of a T-cell malignancy in the patient. The resultant amplified target sequence is detected on gel electrophoresis and compared with a normal sample or standard that does not contain a chromosome 14 abnormality. Virgilio et al., supra, disclose polynucleotide sequences useful as second primers. Other polynucleotide sequences useful as second primers are selected from the T-cell receptor .alpha./delta. locus, the T-cell receptor .beta. chain, or if the chromosome 14 abnormality involves an inversion, a polynucleotide sequence 5' to exon 1 of the *TCL-1b* gene, or if the chromosome abnormality involves a translocation, a polynucleotide sequence 3' to the 3' intron of the *TCL-1b* gene. The amplification of genomic DNA target sequences may require generating long PCR products. PCR techniques for generating long PCR products are described in Science (1994) 263:1564-1565; PCR kits for generating long PCR products are available from Perkin Elmer and Takara Shuzo Co., Ltd. The present invention also provides a method for detecting a target nucleotide sequence indicative of or including at least a portion of a chromosome 14 abnormality in a nucleic acid sample, comprising the steps of hybridizing the sample with a nucleic acid probe of not more than 10 kilobases, comprising in the range of 15-1324 nucleotides complementary to at least a portion of the nucleotide sequence of SEQ ID NO: 11;

and detecting or measuring the amount of any resulting hybridization between the probe and the target sequence within the sample. The resultant hybridization between the probe and the target sequence within the sample is detected using gel electrophoresis and is compared to a target sequence from a normal sample or standard that does not contain a chromosome 14 abnormality. The present invention also provides a method of diagnosing a T-cell malignancy associated with chromosome 14 abnormalities in a patient comprising, detecting said chromosome 14 abnormality according to the method above in which the presence of the amplified target sequence indicates the presence of a T-cell malignancy in the patient. Absolute complementarity between a hybridization probe and a target sequence, although preferred, is not required. A sequence "complementary to at least a portion of", as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the nucleic acid, forming a stable hybridization complex. The ability to hybridize will depend on both the degree of complementarity and the length of the nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *TCL-1b* RNA it may contain and still form a stable duplex (or triplex, as the case is). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

An additional aspect of the present invention relates to diagnostic kits for the detection or measurement of *TCL-1b* gene sequences and Tcl-1b protein. Accordingly, the present invention provides a diagnostic kit comprising, in a container a compound comprising a probe of not more than 10 kilobases and comprising in the range of 15-1324 nucleotides of the nucleotide sequence of SEQ ID NO: 9 or its complement. Alternatively, the present invention provides a diagnostic kit comprising, in one or more containers, a pair of primers of at least 8-25 nucleotides in which at least one of the primers is hybridizable to SEQ ID NO: 9 or its complement and wherein the primers are capable of priming cDNA synthesis in an amplification reaction. The present invention also provides a diagnostic kit in which at least one of the primers is hybridizable to SEQ ID NO: 9 or its complement and in which one of the primers is hybridizable to a DNA sequence

located telomeric or centromeric to the *TCL-1b* gene. In a specific embodiment, one of the foregoing compounds of the container is detectably labeled.

The amplification reaction of the present invention are a polymerase chain reaction, competitive PCR and competitive reverse-transcriptase PCR (Clementi et al., 1994, *Genet Anal Tech Appl*, 11(1):1-6 and Siebert et al., 1992, *Nature*, 359:557-558); cyclic probe reaction, which allows for amplification of a target sequence using a hybrid RNA/DNA probe and RNase (ID Biomedical); ligase chain reaction (Wu, et al., 1989, *Genomics*, 4:560-569). In a particular embodiment, the chromosomal abnormality associated with a *TCL-1b* locus is detected as described in PCT Publication No. WO/92/19775, dated Nov. 12, 1992. In a specific embodiment, the *TCL-1b* probe used in a hybridization assay is detectably labeled. Such a label is any known in the art including, but not limited to, radioactive labels, fluorescent labels, biotin, chemiluminescent labels, etc.

In a specific embodiment in which the assay used employs primers, at least one primer is detectably labeled. In another embodiment, one of a primer pair is attached to a moiety providing for capture, e.g., a magnetic bead.

Anti-*Tcl-1b* antibodies are generated and used diagnostically to detect the presence of *Tcl-1b* protein product in patient samples thereby identifying disease states associated with chromosome 14 abnormalities. For detection of *Tcl-1b* protein sequences, a diagnostic kit of the present invention comprises, in one or more containers, an anti- *Tcl-1b* antibody which optionally is detectably labeled. In a different embodiment, the kit can comprise in a container, a labeled specific binding portion of an antibody. As used herein, the term detectable label refers to any label which provides directly or indirectly a detectable signal and includes, for example, enzymes, radiolabelled molecules, fluorescent molecules, particles, chemiluminesors, enzyme substrates or cofactors, enzyme inhibitors, or magnetic particles. Examples of enzymes useful as detectable labels in the present invention include alkaline phosphatase and horse radish peroxidase. A variety of methods are available for linking the detectable labels to proteins of interest and include, for example, the use of a bifunctional agent, such as, 4,4'-difluoro-3,3'-dinitrophenylsulfone, for attaching an enzyme, for example, horse radish peroxidase, to a protein of interest. The attached enzyme is then allowed to react with a substrate

yielding a reaction product which is detectable. The present invention provides a method for detecting a Tcl -1b protein in a patient sample, comprising, contacting the patient sample with an anti- Tcl -1b antibody under conditions such that immunospecific binding occurs, and detecting or measuring the amount of any
5 immunospecific binding by the antibody.

Samples are any sample from a patient containing Tcl -1b protein, e.g., tissue sections, peripheral blood lymphocytes, etc. In diagnosing disease states, the functional activity of Tcl -1b proteins, derivatives and analogs are assayed by various methods. Accordingly, the present invention also provides a method of
10 diagnosing a T-cell malignancy associated with chromosome 14 abnormalities in a patient comprising, detecting increased expression of Tcl -1b protein in a sample from the patient, in which an increase in Tcl -1b protein relative to the level found in such an analogous sample from a normal individual, indicates the presence of a T-cell malignancy in the patient.

For example, in one embodiment, where one is detecting or measuring Tcl -1b protein by assaying for binding to anti- Tcl -1b antibody, various immunoassays known in the art are used, including, but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays,
20 immunoradiometric assays, gel diffusion precipitin reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope abels, for example), western blots, in situ hybridizations, precipitation reactions, agglutination ssays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and
25 immunoelectrophoresis assays, etc. In one mbodiment, antibody binding is detected by detecting a label on the primary antibody. In nother embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labelled. Many means are known in the art for detecting binding in an immunoassay and are
30 within the scope of the present invention. In particular, such an immunoassay is carried out by a method comprising contacting a sample derived from a patient with an anti- Tcl -1b antibody under conditions such that immunospecific binding occurs,

and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific embodiment, antibody to a Tcl -1b protein is used to assay a patient tissue or serum sample for the presence of a Tcl -1b protein where an increased level of Tcl -1b protein is an indication of a diseased condition. In one
5 embodiment of the present invention, the Tcl -1b protein is detected or measured by immunocytochemistry of a patient sample. In another embodiment, assays to measure the levels of Tcl -1b protein or RNA is used to monitor therapy of disease associated with increased expression of Tcl -1b. For example, a decrease in levels of TCl-1b RNA or protein after therapy, relative to the level found before therapy, are
10 indicative of a favorable response to therapy. An increase in such levels after therapy are indicative of a poor response to therapy.

In another embodiment, the levels of Tcl -1b protein or RNA expression are used to stage disease, with an increase in Tcl -1b protein or RNA expression indicating disease progression.

15 Other methods will be known to the skilled artisan and are within the scope of the invention.

Therapeutic/Prophylactic Uses

20 Inhibitors of Tcl-1b are used therapeutically for the treatment of disease states associated with chromosome 14 abnormalities, in particular at 14q32.1, and/or increased expression of Tcl -1b protein. In an embodiment of the present invention, a Tcl -1b protein and/or cell line that expresses a Tcl -1b protein is used to screen for antibodies, peptides, or other molecules that bind to the Tcl -1b protein
25 and thus may act as agonists or antagonists of Tcl -1b protein. For example, anti-Tcl -1b antibodies capable of neutralizing the activity of a Tcl -1b protein are used to inhibit or prevent a disease state associated with chromosome 14 abnormalities and/or expression of Tcl -1b protein, such as T-cell leukemia and lymphoma. Accordingly, the present invention provides a method for treating a disease state
30 associated with a chromosome 14 abnormality in mammal suffering from a disease state associated with a chromosome 14 abnormality comprising, administering a therapeutically effective amount of an anti- Tcl -1b antibody to a mammal suffering

from a disease state associated with a chromosome 14 abnormality. Alternatively, screening of organic or peptide libraries with recombinantly expressed Tcl -1b protein are useful for identification of therapeutic molecules that function to inhibit the activity of Tcl -1b protein. Synthetic and naturally occurring products are
5 screened in a number of ways deemed routine to those of skill in the art.

The ability of antibodies, peptides or other molecules to modulate the effect of Tcl -1b protein on disease states is monitored. For example, the expression of TCL-1b gene sequences or Tcl -1b protein sequences are detected as described, supra, both before and after administration of a therapeutic composition comprising
10 a TCL-1b nucleotide sequence, Tcl -1b protein sequence, derivative or analog thereof, or antibody thereto, of the present invention.

A TCL-1b polynucleotide is useful in the treatment of various disease states associated with chromosome 14 abnormalities, such as T-cell leukemias and lymphomas, and/or increased expression of Tcl-1b protein. By introducing TCL-1b
15 antisense gene sequences into cells, gene therapy is used to treat conditions associated with over-expression of TCL-1b genes. Accordingly, the present invention provides a method for treating a disease state associated with a chromosome 14 abnormality in mammal suffering from a disease state associated with a chromosome 14 abnormality comprising, administering a therapeutically
20 effective amount of a TCL-1b antisense molecule to a mammal suffering from a disease state associated with a chromosome 14 abnormality.

Oligonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes that function to inhibit the translation of a *TCL-1b* mRNA are within the scope of the invention. "Antisense" as used herein refers to a nucleic acid
25 capable of hybridizing to a portion of a *TCL-1b* RNA (preferably mRNA) by virtue of some sequence complementarity. Antisense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between
30 -10 and +10 regions of a *TCL-1b* nucleotide sequence, are preferred. The present invention provides for an antisense molecule comprising a nucleotide sequence complementary to at least a part of the coding sequence of a Tcl-1b protein which is

hybridizable to a *TCL-1b* mRNA. The present invention also provides for an antisense molecule with a nucleotide sequence complementary to at least a part of the non-coding sequence (SEQ ID NO: 11) which hybridizes to the *TCL-1b* coding sequence (SEQ ID NO: 11). In a preferred embodiment of the present invention, the antisense gene sequence is derived from the 5' non-coding sequence of a *TCL-1b* gene. In a particularly preferred embodiment of the present invention, the antisense gene sequence is derived from SEQ ID NO: 9.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of *TCL-1b* RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site are evaluated for predicted structural features, such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention are prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules are generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences are incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA

constitutively or inducibly, depending on the promoter used, is introduced stably into cell lines.

Various modifications to the DNA molecules are introduced as a means of increasing intracellular stability and half-life. Examples of modifications include, but are not limited to, the addition of flanking sequences of ribo- or deoxy-nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Methods for introducing nucleic acid into cells or tissue include methods for in vitro introduction of nucleic acid such as the insertion of naked nucleic acid, i.e., by injection into tissue, the introduction of a nucleic acid in a cell *ex vivo*, the use of a vector such as a virus, retrovirus, phage or plasmic, etc. or techniques such as electroporation which are *used in vivo* or *ex vivo*.

Other methods will be known to the skilled artisan and are within the scope of the invention.

Demonstration of Therapeutic or Prophylactic Utility

The TCL-1b polynucleotides, Tcl-1b protein products, derivatives and analogs thereof, and antibodies thereto, of the invention are tested *in vivo* for the desired therapeutic or prophylactic activity. For example, such compounds are tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art are used.

Therapeutic/Prophylactic Methods and Compositions

The invention provides methods of treatment and prophylaxis by administration to a subject of an effective amount of a Therapeutic, i.e., a TCL-1b polynucleotide, Tcl-1b protein, derivative or analog thereof, or antibody thereto of the present invention. In a preferred aspect, the Therapeutic is substantially purified.

The subject is preferably an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and used to administer a Therapeutic of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, expression by recombinant cells, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J Biol Chem*, 262:4429-4432), construction of a therapeutic nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, and oral routes. The compounds are administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and are administered together with other biologically active agents. Administration is systemic or local. In addition, it are desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection are facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

In a specific embodiment, it are desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this are achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration is by direct injection at the site (or former site) of a malignant tumor or neoplastic or pre-neoplastic tissue.

In a specific embodiment where the Therapeutic is a nucleic acid encoding a protein therapeutic, the nucleic acid is administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating

with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliet et al., 1991, *Proc Natl Acad Sci USA*, 88:1864-1868), etc. Alternatively, a nucleic acid therapeutic is introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a therapeutic, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition are sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition is a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition is formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation includes standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it is dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline is provided so that the ingredients are mixed prior to administration.

The Therapeutics of the invention are formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the Therapeutic of the invention which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro assays are employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses are extrapolated from dose-response curves derived from in vitro or animal model test systems.

Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Antisense Regulation of *TCL-1b* Gene Expression

The present invention provides the therapeutic or prophylactic use of nucleic acids of at least six nucleotides that are antisense to a *TCL-1b* gene (SEQ. ID. NO: 11) or cDNA (SEQ. ID. NO: 9) encoding Tcl-1b or a portion thereof. Such

antisense nucleic acids have utility as Antagonist Therapeutics of the invention, and is used in the treatment or prevention of disorders, e.g., T-cell malignancies as described *supra*.

5 The antisense nucleic acids of the invention are oligonucleotides that are double-stranded or single-stranded, RNA or DNA or a modification or derivative thereof, which can be directly administered to a cell, or which are produced intracellularly by transcription of exogenous, introduced sequences.

10 In a specific embodiment, the TCL-1b antisense polynucleotides provided by the instant invention can be used for the treatment of disease states associated with chromosome 14 abnormalities, in particular at 14q32.1, wherein the disease state can be demonstrated (in vitro or in vivo) to express the TCL-1b gene. Such demonstration can be by detection of *TCL-1b* RNA or of Tcl-1b protein.

15 The invention further provides pharmaceutical compositions comprising an effective amount of the TCL-1b antisense nucleic acids of the invention in a pharmaceutically acceptable carrier, as described *supra*. Methods for treatment and prevention of disease states associated with chromosome 14, such as T-cell malignancies comprising administering the pharmaceutical compositions of the invention are also provided.

20 In another embodiment, the invention is directed to methods for inhibiting the expression of a TCL-1b nucleic acid sequence in a prokaryotic or eukaryotic cell comprising providing the cell with an effective amount of a composition comprising an antisense TCL-1b nucleic acid of the invention.

25 The TCL-1b antisense polynucleotides are of at least six nucleotides and are preferably oligonucleotides (ranging from 6 to about 50 oligonucleotides). In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 20 nucleotides, at least 30 nucleotides, or at least 40 nucleotides. The oligonucleotides are DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide is modified at the base moiety, sugar moiety, or phosphate backbone. The oligonucleotide may include
30 other appending groups such as peptides, or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, *Proc Natl Acad Sci USA*, 86:6553-6556; Lemaitre et al., 1987, *Proc Natl Acad Sci USA*, 84:648-652; PCT Publication

No. WO 88/09810, published Dec. 15, 1988) or blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134, published Apr. 25, 1988), hybridization-triggered cleavage agents (see, e.g., Krol et al., 1988, *BioTechniques*, 6:958-976) or intercalating agents (see, e.g., Zon, 1988, *Pharm Res*, 5:539-549).

5 The oligonucleotide are conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

Oligonucleotides of the invention are synthesized by standard methods known in the art, e.g., by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, 10 phosphorothioate oligos are synthesized by the method of Stein et al. (1988, *Nucl. Acids Res.* 16:3209), methylphosphonate oligos are prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, *Proc Natl Acad Sci USA*, 85:7448-7451), etc.

15 In a specific embodiment, the TCL-1b antisense oligonucleotide comprises catalytic RNA, or a ribozyme (see, e.g., PCT International Publication WO 90/11364, published Oct. 4, 1990; Sarver et al., 1990, *Science*, 247:1222-1225). In another embodiment, the oligonucleotide is a 2'-O-methylribonucleotide (Inoue et al., 1987, *Nucl. Acids Res.* 15:6131-6148), or a chimeric RNA-DNA analogue 20 (Inoue et al., 1987, *FEBS Lett*, 215:327-330).

In an alternative embodiment, the TCL-1b antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector is introduced in vivo such that it is taken up by a cell, within which cell the vector or a portion thereof is transcribed, producing an antisense 25 nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the TCL-1b antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it is transcribed to produce the desired antisense RNA. Such vectors are constructed by recombinant DNA technology methods standard in the art. Vectors are plasmid, viral, or others known in the art, 30 used for replication and expression in mammalian cells. Expression of the sequence encoding the TCL-1b antisense RNA is by any promoter known in the art to act in mammalian, preferably human, cells. Such promoters are inducible or constitutive.

Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, *Nature* 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., 1980, *Cell*, 22:787-797), the herpes thymidine kinase promoter (Wagner et al., 1981, *Proc Natl Acad Sci USA*, 78:1441-1445), the regulatory sequences of the metallothionein gene (Brinster et al., 1982, *Nature*, 296:3942), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a *TCL-1b* gene, preferably a human *TCL-1b* gene. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded *TCL-1b* antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation are assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with a *TCL-1b* RNA it may contain and still form a stable duplex (or triplex, as the case are). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

The *TCL-1b* antisense nucleic acids are used to treat (or prevent) T-cell malignancies, of a cell type which has been shown to express *TCL-1b* RNA. Malignant, neoplastic, and pre-neoplastic cells which are tested for such expression include, but are not limited, to those described supra. In a preferred embodiment, a single-stranded DNA antisense *TCL-1b* oligonucleotide is used.

Malignant (particularly, tumor) cell types which express *TCL-1b* RNA is identified by various methods known in the art. Such methods include but are not limited to hybridization with a *TCL-1b*-specific nucleic acid (e.g., by Northern hybridization, dot blot hybridization, in situ hybridization), observing the ability of RNA from the cell type to be translated *in vitro* into *TCL-1b*, etc. In a preferred aspect, primary tumor tissue from a patient is assayed for *TCL-1b* expression prior to treatment.

Pharmaceutical compositions of the invention, comprising an effective amount of a TCL-1b antisense nucleic acid in a pharmaceutically acceptable carrier, is administered to a patient having a malignancy which is of a type that expresses *TCL-1b* RNA.

5 The amount of TCL-1b antisense nucleic acid which will be effective in the treatment of a particular disease state or condition will depend on the nature of the disease state or condition, and is determined by standard clinical techniques. Where possible, it is desirable to determine the antisense cytotoxicity of the tumor type to be treated *in vitro*, and then in useful animal model systems prior to testing and use
10 in humans.

 In a specific embodiment, pharmaceutical compositions comprising TCL-1b antisense nucleic acids are administered via liposomes, microparticles, or microcapsules. In various embodiments of the invention, it are useful to use such compositions to achieve sustained release of the TCL-1b antisense nucleic acids. In
15 a specific embodiment, it are desirable to utilize liposomes targeted via antibodies to specific identifiable tumor antigens (Leonetti et al., 1990, *Proc Natl Acad Sci USA*, 87:2448-2451; Renneisen et al., 1990, *J Biol Chem*, 265:16337-16342).

CLAIMS

What is claimed is:

- 5 1. An isolated nucleic acid comprising a nucleotide sequence encoding a Tcl-1b protein, wherein said nucleotide sequence is a cDNA sequence.
- 10 2. The isolated nucleic acid of claim 1, wherein said nucleotide sequence encodes a human Tcl-1b protein having an amino acid sequence of SEQ ID NO: 10 from amino acid number 1 to 128.
- 15 3. An isolated nucleic acid of not more than 50 kilobases which contains at least an 18 nucleotide portion encoding a Tcl-1b protein fragment.
- 20 4. An isolated nucleic acid of not more than 50 kilobases which contains at least an 18 nucleotide portion of the sequence depicted in SEQ ID NO: 11
- 25 5. The isolated nucleic acid of claim 1, comprising a nucleotide sequence of SEQ ID NO: 9 from nucleotide number 1 to 1152.
- 30 6. A Tcl-1b protein.
7. The isolated Tcl-1b protein of claim 6, comprising an amino acid sequence of SEQ. ID. NO: 10 from amino acid 1-128.
8. An isolated nucleic acid, comprising a sequence encoding a fragment of a protein having an amino sequence of SEO ID NO: 10 from amino acid number 1 to 128, which fragment can be specifically bound by an antibody to a Tcl-1b protein.

9. A recombinant DNA vector, comprising a nucleotide sequence that encodes a Tcl-1b protein, wherein said nucleotide sequence is a cDNA sequence.

5

10. A host cell that contains said recombinant DNA vector of claim 7.

10

11. The recombinant DNA vector of claim 7, wherein the nucleotide sequence encodes a human Tcl-1b protein having an amino acid sequence of SEQ ID NO: 10 from amino acid number 1 to 128.

15

12. An isolated nucleic acid of not more than 50 kilobases which contains at least a 50 nucleotide portion of SEQ ID NO: 11.

20

13. An isolated nucleic acid that is capable of hybridizing under stringent conditions to a nucleotide sequence that is complementary to the cDNA sequence of SEQ ID NO: 9, said nucleic acid containing at least an 25 nucleotide portion of SEQ ID NO: 9.

25

14. An isolated nucleic acid that is capable of hybridizing under stringent conditions to a nucleotide sequence that is complementary to a cDNA sequence that encodes a Tcl-1b protein, which protein has an amino acid sequence of SEQ ID NO: 10, and said nucleic acid containing at least an 25 nucleotide portion of SEQ ID NO: 9.

30

15. An antisense molecule, comprising a nucleotide sequence complementary to at least a part of a coding sequence of a Tcl-1b protein, which is hybridizable to a Tcl-1b mRNA.

16. The antisense molecule of claim 15, wherein said nucleotide sequence is complementary to a least a part of the sequence depicted in SEQ. ID. NO: 9.

5 17. A fusion protein comprising a Tcl-1b protein sequence of at least 10 amino acids linked to a non-Tcl-1b protein sequence.

18. An antibody which binds to an epitope of a Tcl-1b protein.

10 19. An isolated protein comprising an amino acid sequence having at least 70% amino acid sequence identity to an amino acid sequence depicted in SEQ. ID. NO: 10, over a contiguous sequence of at least 25 amino acids.

15 20. A method for detecting a target sequence indicative of a chromosome 14 abnormality in a sample, comprising the steps of:

a) amplifying said target sequence in said sample using a first primer of 18 to 25 nucleotides complementary to a nucleotide sequence of SEQ. ID. NO: 9, and a second primer complementary to a region telomeric or centromeric, preferably from a T-cell receptor α/δ locus, to said Tcl-1b gene; and

20 b) detecting any resulting amplified target sequence in which the presence of said amplified target sequence is indicative of said chromosome 14 abnormality.

25 21. The method of claim 20, wherein said chromosome 14 abnormality is in a Tcl-1b locus and comprises a t(14:14)(q11;q32) translocation or an inv (14)(q11;q32) inversion.

22 A host cell that contains a recombinant vector comprising a cDNA sequence that encodes a human Tcl-1b protein having the amino acid sequence of SEQ ID NO: 10 from amino acid number 1 to 128.

5 23. A host cell that contains a recombinant vector comprising a nucleic acid that is capable of hybridizing under stringent conditions to a nucleotide sequence that is complementary to a cDNA sequence that encodes a Tcl-1b protein, which protein has the amino acid sequence of SEQ ID NO: 10, and said nucleic acid containing at least
10 an 25 nucleotide portion of SEQ ID NO: 9.

25. A pharmaceutical composition, comprising said antisense molecule of claim 15 or 16 in a pharmaceutically acceptable carrier.

15 26. A pharmaceutical composition, comprising said antibody of claim 18 in a pharmaceutically acceptable carrier.

27. A method for detecting a target nucleotide sequence indicative of a chromosome 14 abnormality in a nucleic acid sample, comprising
20 the steps of:

a) hybridizing said sample with a nucleic acid probe of not more than 10 kilobases, comprising in the range of 15-1152 nucleotides complementary to said nucleotide sequence of SEQ. ID. NO: 9; and

25 b) detecting or measuring an amount of any resulting hybridization between said probe and said target sequence within said sample.

28. The method of claim 27, wherein said chromosome 14
30 abnormality is in a Tcl-1b locus and comprises a t(14:14)(q11:q32) translocation or an inv (14)(q11:q32) inversion.

29. A method for detecting a Tcl-1b protein in a patient sample, preferably a human sample, comprising:

- a) contacting said patient sample with an anti-Tcl-1b antibody under conditions such that immunospecific binding occurs; and
- b) detecting or measuring an amount of any immunospecific binding by said antibody.

30. A diagnostic kit, comprising in one or more containers, a pair of primers, each having at least 15-25 nucleotides, in which at least one of said primers is hybridizable to SEQ. ID. NO: 9 or its complement and wherein said primers are capable of priming DNA synthesis in a nucleic acid amplification reaction.

31. A method for treating a disease state associated with a chromosome 14 abnormality in a mammal, preferably a human, suffering from said disease state associated with said chromosome 14 abnormality, comprising administering a therapeutically effective amount of a Tcl-1b antisense molecule or an anti-Tcl-1b antibody to said mammal.

32. The method of claim 31, wherein said disease state comprises a T-cell leukemia or lymphoma and said chromosome 14 abnormality comprises a t(14:14)(q11:q32) translocation or an inv (14)(q11:q32) inversion.

1/4

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TCL1	47	RVLELRREDVVLGRP	PQGPSLLP	MWQLYPGRYSSDSSEWR
MTCP1	42	ARV..QQQVPLGAARP	HILTSQLP	MWQLYPERYLDNISRRLMQLQHHL
TCL1b	59	TVHLWQAVHTR	LSCQPTFSQLPN	WQLYPGRYRAADSSEWEIADHGQIDSTEQ
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Fig. 1

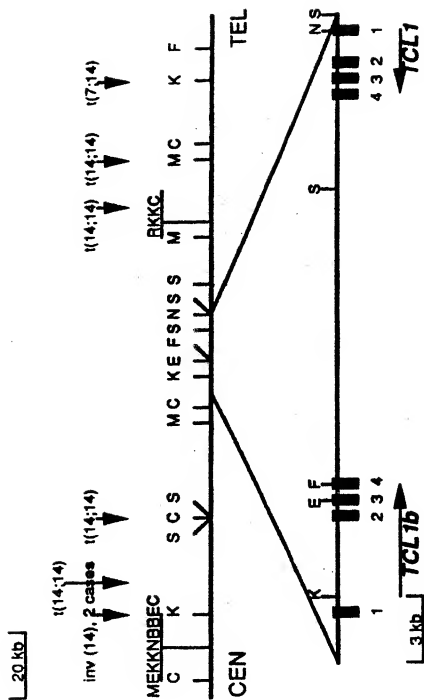


Fig. 2

3/4

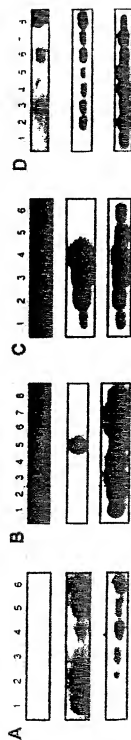


Fig. 3



Fig. 4

SEQUENCE LISTING

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6486

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/06612

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : Please See Extra Sheet.

US CL : 536/24.5; 530/387.1, 387.9; 435/4, 7.1, 7.21, 7.23; 514/2, 44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/24.5; 530/387.1, 387.9; 435/4, 7.1, 7.21, 7.23; 514/2, 44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS, CANCERLIT, BIO. AND AGRI. INDEX, CUR. BIOTECH AB., CLAIMS/U.S. PAT. AB., CHINESE PAT. AB., EUROPEAN PAT., DERWENT, IMSWORLD PAT., EMBASE, MEDLINE, WIPO/PCT PATENTS, DIALOG, EAST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CONDORELLI et al. T-Cell-directed <i>TAL-1</i> Expression induces T-Cell Malignancies in Transgenic Mice. Cancer Research. 15 November 1996, Vol. 56, pages 5113-5119.	29



Further documents are listed in the continuation of Box C.



See patent family annex.

*

Special categories of cited documents:

A

document defining the general state of the art which is not considered to be of particular relevance

E

earlier document published on or after the international filing date

L

document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O

document referring to an oral disclosure, use, exhibition or other means

P

document published prior to the international filing date but later than the priority date claimed

**

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

**X*

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

**Y*

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

A

document member of the same patent family

Date of the actual completion of the international search

04 MAY 2000

Date of mailing of the international search report

19 MAY 2000

Name and mailing address of the ISA/US

Commissioner of Patents and Trademarks

Box PCT

Washington, D.C. 20231

Facsimile No. (703) 305-3230

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US00/06612

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

C07H 21/00, 21/04; C07K 16/00, 17/00, 17/02, 17/04; G01N 33/53, 33/574; A61K 38/00, 38/04, 38,08, 38/16, 48/00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/06612**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-14, 17, 19-24, 27, 28 and 30
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

The search on these independent claims, as well as their dependent claims could not be conducted because the CRF for this case is defective.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.